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Anti-PL-7, anti-Ro/SSA, anti-Mi-2, and anti-TIF1-γ correlate with specific patterns of histopathologic features in dermatomyositis: An analysis of 39 skin biopsy specimens from 25 patients

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Abstract

Background: In dermatomyositis (DM), myositis-specific and myositis-associated antibodies have been correlated with clinical features. It is unknown if histopathologic findings in lesional skin biopsies correlate with serologic subtypes of DM.

Methods: A retrospective chart review of patients with DM was performed. Patients with myositis antibodies and DM lesional skin biopsies were included in the study. Skin biopsies were reviewed by blinded dermatopathologists for 20 histopathologic features.

Results: There was a statistically significant ($p < 0.05$) association between anti-PL-7 serology and decreased degree of vacuolar degeneration, necrotic keratinocytes, and thickening of the epidermal basement membrane. Anti-aminoacyl tRNA synthetase (anti-ARS) antibodies had the same significant negative association with degree of vacuolar degeneration, necrotic keratinocytes, and thickening of the epidermal basement membrane. A similar pattern was seen with an anti-cytoplasmic serology; where there was a significant association with an increased degree of vacuolar degeneration and necrotic keratinocytes, and a nonsignificant trend of minimally thickened epidermal basement membrane. There was a statistically significant association between anti-Ro/SSA serology and increased degree of vacuolar degeneration. Anti-TIF1-γ serology was significantly associated with the increased presence of necrotic keratinocytes and pigment incontinence, and displayed a pattern of increased neutrophils. There was a significant association between anti-Mi-2 antibodies and pigment incontinence, as well as between myositis-specific antibodies and pigment incontinence. A statistically significant positive association was found between nuclear antibodies and degree of vacuolar degeneration, thickened epidermal basement membrane, pigment incontinence, and epidermal atrophy.

Conclusion: In patients with DM, some specific serotypes, including anti-PL-7, anti-Ro/SSA, anti-Mi-2, and anti-TIF1-γ, may have characteristic histopathologic features.

KEYWORDS
anti-Mi-2, anti-PL-7, anti-Ro/SSA, anti-TIF1-γ, dermatomyositis, myositis-associated antibodies (MAAs), myositis-specific antibodies (MSAs), serology

1 | INTRODUCTION

Dermatomyositis (DM) is an inflammatory autoimmune disorder with characteristic cutaneous findings and heterogenous clinical presentations that can include myositis, interstitial lung disease (ILD), and malignancy. DM-associated ILD is likely to be refractory to corticosteroid treatment alone, with 5-year survival rates as low as 55.6%.[1] Patients with DM have a threefold increased risk of malignancy. Thus, for many patients, timely and accurate diagnosis of DM can mean prompt cancer treatment. Cancers associated with DM include ovarian, lung, pancreatic, stomach, and colorectal cancers, as well as non-Hodgkin lymphoma.[2]

Cutaneous findings highly specific for DM include violaceous papules and plaques on the joints of the hands (Gottron papules), erythematous macules or patches on the extensor surfaces of the knees, elbows, ankles, or knuckles (Gottron sign), and periorbital edema and erythema involving the upper eyelids (Heliotrope rash), erythematous macules or patches over the upper trunk distributed in a "shawl pattern" (Shawl sign), and symmetric poikiloderma over the lateral hips and thighs (Holster sign).[3] Histopathologic features of DM can be subtle and depend on the age and site of the lesion. The typical histologic picture is a superficial perivascular and interstitial dermatitis with vacuolar interface changes, ectatic vessels, few necrotic keratinocytes, and sometimes an increase in interstitial mucin.[4]

Serologic studies reveal autoantibodies in up to 80% of DM patients.[5–8] These autoantibodies are classified as either myositis-specific antibodies (MSAs) or myositis-associated antibodies (MAAs) (Table 1). MSAs are over 90% specific for DM.[9] The anti-aminoacyl tRNA synthetase (anti-ARS) antibodies are the most common MSAs. Anti-ARS antibodies are associated with anti-synthetase syndrome, which may include ILD, myositis, nonerosive arthritis, Raynaud's phenomenon, fever, and hyperkeratosis on the hands ("mechanic's hands").[10] Other DM-specific antibodies are associated with malignancy, such as anti-TIF1-γ and anti-NXP-2.[11,12] This is in contrast to DM autoantibodies such as anti-Mi-2, where there is a decreased likelihood of developing malignancy or ILD.[11]

In contrast, MAAs are present in up to 50% of DM patients, but are not specific for DM, and are often seen in myositis-connective tissue disease overlap syndromes. Autoantibodies can also be classified based on whether they target an antigen in the nucleus, cytoplasm, or nucleolus (see Table 2). Antinuclear antibodies and anti-cytoplasmic antibodies are serological markers used in the diagnosis of systemic autoimmune connective tissue diseases.[13]

The clinical phenotype and sequelae related to MSA and MAA are a growing area of research that may impact the clinical management of DM. However, as the value of MSA and MAA in clinical monitoring is still being characterized, screening for malignancy and ILD is recommended in all patients with DM, regardless of serology.[14]

While various clinical and serotype correlations have been investigated, to our knowledge, only one study has previously examined correlations between serotype and the dermatopathological findings of DM.[3,9,15,16] In this pilot study, blinded assessors analyzed the histopathologic features of lesional skin biopsies from a cohort of patients with clinically confirmed and seropositive DM, and the data were analyzed for correlations between histopathologic features and specific serotypes.

2 | MATERIALS AND METHODS

Approval was granted by the institutional review board (IRB) and the consent process was waived. We conducted a retrospective search of electronic medical records to identify all adult patients with a diagnosis of DM who were evaluated or treated at a Northeastern academic referral center between January 1, 2000, and November 4, 2022. Patients were considered for inclusion in the study if they had a positive myositis antibody panel and at least one surgical pathology report for a DM-associated lesional skin biopsy performed at the medical

TABLE 1 Myositis-specific antibodies and myositis-associated antibodies that may be present in patients with dermatomyositis.

Myositis-specific antibodies	Myositis-associated antibodies
Anti-ARS,[a] anti-SRP, anti-MDA-5, anti-Mi-2, anti-TIF1-γ, anti-SAE1	ANA, anti-Ro/SSA, anti-PM-Scl, anti-KU, anti-U2 snRNP

[a]Anti-aminoacyl tRNA synthetase (anti-ARS) includes anti-Jo-1, PL-7, PL-12, EJ, OJ, KS, Ha, and Zo.

TABLE 2 Myositis antibodies characterized according to location of antigen.

Nuclear	Cytoplasmic	Nucleolar
Anti-Mi-2, TIF1-γ, anti-SAE, anti-NXP2	Anti-SRP, anti-ARS,[a] anti-HMGCR, anti-MDA5	Anti-PM-Scl, anti-U3RNP, anti-RuvBL1/2

[a]Anti-aminoacyl tRNA synthetase (anti-ARS) includes anti-Jo-1, PL-7, PL-12, EJ, OJ, KS, Ha, Zo.

center. Seronegative DM patients were not used as negative controls, since they could have DM autoantibodies that were uncharacterized at the time of serologic testing. A board-certified dermatopathologist reviewed all skin biopsy slides to determine if they met the study criteria. Since the focus of the study was on dermatitis, one case of DM panniculitis was excluded. This left 25 patients with 39 skin biopsy samples to be included in the study. Of these 25 patients, 11 had more than one biopsy (Table S1). The findings of the first positive myositis antibody panel were collected for each of these patients (Table S1). For patients with multiple positive myositis antibodies, clinically relevant MSAs were selected over MAAs as the dominant antibody. After, the antibody with the highest titer was considered the dominant antibody.

A panel of five board-certified dermatopathologists, and one board-eligible dermatopathology fellow, reviewed the skin biopsies. To help ensure generalizability, the panel was composed of dermatopathologists with a broad range of experience (<1 year to 15 years). All were blinded to serologic status, clinical presentation, and medical history. For each case, the presence or degree of 20 histopathologic features common in DM was tabulated (Table 3). Because the quantification of features such as the number of leukocytes in an inflammatory infiltrate can be highly unreliable, we employed a binary or simple graded classification system to minimize interobserver variability where possible.[17] All features of all biopsies were reviewed three times, independently, by three different dermatopathologists. For categorical variables, the mode of the findings reported by the three dermatopathologists was recorded as the final response. For continuous variables, the mean was recorded as the final response.

We analyzed the data for associations between serotype and histopathologic features using R (R version 3.6.1, R Core Team, Vienna, Austria). We tested the association between each continuous variable and each of the serotypes using logistic regression, then confirmed these findings using Wilcoxon rank-sum test. To test the association between each of the binary predictors and each serotype, we used Fisher's exact test. All reported *p*-values have not been adjusted for multiple hypothesis testing because this is a hypothesis-generating study, and such adjustments can mask true signals in such studies.

We considered whether, in some cases, it may be true that no correlation exists between histopathology and individual MSA or MAA, but there may be a correlation between histopathology and serologic groups. Thus, we also grouped serotypes according to two existing systems of classification (Table 4). The first categorized serotypes as anti-ARS, myositis-specific, or myositis-associated antibodies.[18] The second classified each serotype as targeting cytoplasmic, nucleolar, or nuclear antigens.[13] We tested the association between each feature and each of these six outcomes using the same statistical methods described above.

DM patients positive for anti-Mi-2, anti-SAE, or anti-TIF1-γ were included in the antinuclear antibody category. Anti-NXP patients would have also been included, but there were none in our patient sample. MSA includes anti-Mi-2, anti-TIF1-γ, anti-SAE1, anti-MDA-5, and anti-ARS antibodies. Anti-SRP is also included, but there were none in our patient sample.

TABLE 3 Histopathologic features evaluated in lesional skin biopsy slides of patients with dermatomyositis.

Feature	Rating system
Density of the inflammatory infiltrate[a]	0—Sparse 1—Dense
Lichenoid or vacuolar[a]	0—Vacuolar 1—Lichenoid
Degree of vacuolar degeneration[a]	0—Absent 1—Present, minimal 2—Present, marked
Predominantly adnexotropic infiltrate[b]	0—Absent 1—Present
Corneal layer alterations[a]	0—Basket weave 1—Focally altered 2—Altered 3—Hyperkeratosis
Epidermal atrophy[b]	0—Absent 1—Present
Acanthosis[b]	0—Absent 1—Present
Spongiosis[b]	0—Absent 1—Present
Effacement of the rete[b]	0—Absent 1—Present
Necrotic keratinocytes[a]	0—Absent 1—Present, minimal 2—Present, marked
Thickened epidermal basement membrane[a]	0—Absent 1—Present, minimal (i.e., involves <1/2 the section) 2—Present, marked (≥1/2 the section)
Ectatic vessels[b]	0—Absent 1—Present
Thickened vascular basement membrane[a]	0—Absent 1—Present, minimal 2—Present, marked
Increased interstitial mucin[a]	0—Absent 1—Present, minimal 2—Present, marked
Pigment incontinence[a]	0—Absent 1—Few (<5/HPF) 2—Numerous (≥5/HPF)
Eosinophils[b]	0—Absent 1—Present
Neutrophils[b]	0—Absent 1—Present
Plasma cells[b]	0—Absent 1—Present
Extravasated RBCs[b]	0—Absent 1—Present
Nuclear dust[b]	0—Absent 1—Present

Abbreviations: HPF, high power field; RBCs, red blood cells.
[a]Continuous variable.
[b]Categorical variable.

3 | RESULTS

There were 10 unique serotypes present in the cohort. The average Fleiss kappa was 0.35 across all features. All statistical tests on continuous variables, based on a logistic regression model, were also confirmed by the Wilcoxon rank-sum test. We assessed the association between binary features and individual serotypes using Fisher's exact test (Table 7). To analyze associations between continuous features and grouped serotypes, we used logistic regression (Table 6).

3.1 | Continuous variables

3.1.1 | Anti-Mi-2

There was a statistically significant ($p < 0.05$) association between anti-Mi-2 serology and pigment incontinence (odds ratio [OR], 5.67; 95% confidence interval [CI], 1.04–31.0) (Table 5).

TABLE 4 Summary of counts of individual and grouped serotypes.

Antibody	Count
Anti-PL-7	7
Anti-SSA	6
Anti-SAE1	6
Anti-PM/Scl-100	5
Anti-Mi-2	5
Anti-TIF1-γ	4
Anti-KU	3
Anti-EJ	1
Anti-MDA-5	1
Anti-U2 RNP	1
Classification System 1	
Anti-synthetase antibodies	8
Myositis-specific antibodies	16
Myositis-associated antibodies	15
Classification System 2	
Cytoplasmic antibodies	9
Nucleolar antibodies	5
Nuclear antibodies	25

3.1.2 | Anti-PL-7

There was a statistically significant ($p < 0.05$) association between serotype anti-PL-7 and decreased degree of vacuolar degeneration (OR, 0.01; 95% CI, 0.00–0.36), fewer necrotic keratinocytes (OR, 0.05; 95% CI, 0.00–0.60), and minimally thickened epidermal basement membrane (OR, 0.09; 95% CI, 0.01–0.87) (Table 5).

3.1.3 | Anti-Ro/SSA

There was a statistically significant ($p < 0.05$) association between increased degree of vacuolar degeneration (OR, 19.5; 95% CI, 1.78–212.0) and anti-Ro/SSA serology (Table 5).

3.1.4 | Anti-TIF1-γ

There was a statistically significant association between increased presence of necrotic keratinocytes (OR, 41.2; 95% CI, 1.64–1038.0) and anti-TIF1-γ serology (Table 5).

3.1.5 | Anti-synthetase antibodies

There was a statistically significant ($p < 0.05$) association of anti-ARS antibodies with decreased degree of vacuolar degeneration (OR, 0.01; 95% CI, 0.00–0.42), fewer necrotic keratinocytes (OR, 0.04; 95% CI, 0.00–0.45), and minimally thickened epidermal basement membrane (OR, 0.09; 95% CI, 0.01–0.76) (Table 6).

3.1.6 | Myositis-specific antibodies

There was a statistically significant ($p < 0.05$) association between MSAs and pigment incontinence (OR, 3.33; 95% CI, 1.09–10.1) (Table 6).

3.1.7 | Anti-cytoplasmic antibodies

We found a statistically significant ($p < 0.05$) association between anti-cytoplasmic antibodies and decreased degree of vacuolar

TABLE 5 Association between continuous features and individual serotypes as determined by logistic regression.

	Anti-M1-2			Anti-PL-7			Anti-SSA			Anti-TIF1-γ		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Degree of vacuolar degeneration	8.43	(0.87, 81.8)	0.066	0.01	(0.00, 0.36)	0.014*	19.5	(1.78, 212.0)	0.015*	3.46	(0.33, 35.9)	0.298
Necrotic keratinocytes	3.53	(0.48, 26.0)	0.217	0.05	(0.00, 0.60)	0.018*	1.09	(0.19, 6.33)	0.926	41.2	(1.64, 1038.0)	0.024*
Thickened epidermal basement membrane	2.20	(0.29, 16.8)	0.449	0.09	(0.01, 0.87)	0.037*	1.47	(0.23, 9.42)	0.682	11.8	(0.81, 173.0)	0.071
Pigment incontinence	5.67	(1.04, 31.0)	0.045*	0.16	(0.02, 1.30)	0.086	1.63	(0.43, 6.20)	0.475	8.92	(1.08, 73.9)	0.043*

Note: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
Abbreviations: CI, confidence interval; OR, odds ratio.

TABLE 6 Association between continuous features and grouped serotypes as determined by logistic regression.

	Classification System 1						Classification System 2									
	Anti-synthetase antibodies			Myositis-specific antibodies			Myositis-associated antibodies			Cytoplasmic antibodies			Nucleolar antibodies			Nuclear antibodies		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Degree of vacuolar	0.01	(0.00, 0.42)	0.014*	2.89	(0.59, 14.1)	0.188	2.08	(0.44, 9.75)	0.354	0.04	(0.00, 0.67)	0.025*	0.71	(0.07, 6.95)	0.755	10.9	(1.26, 94.2)	0.030*
Necrotic keratinocytes	0.04	(0.00, 0.45)	0.010**	4.27	(0.97, 18.8)	0.055	1.30	(0.35, 4.84)	0.694	0.13	(0.02, 0.90)	0.039*	2.52	(0.36, 17.6)	0.351	2.46	(0.60, 10.1)	0.213
Thickened epidermal basement membrane	0.09	(0.01, 0.76)	0.028*	3.85	(0.84, 17.7)	0.084	1.13	(0.29, 4.46)	0.858	0.16	(0.02, 1.04)	0.055	0.25	(0.03, 2.22)	0.216	10.0	(1.56, 65.0)	0.015*
Pigment incontinence	0.17	(0.03, 1.16)	0.071	3.33	(1.09, 10.1)	0.034*	0.74	(0.26, 2.09)	0.566	0.29	(0.06, 1.33)	0.110	0.28	(0.04, 2.18)	0.225	4.66	(1.20, 18.1)	0.026*

Note: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
Abbreviations: CI, confidence interval; OR, odds ratio.

degeneration (OR, 0.04; 95% CI, 0.00–0.67) and fewer necrotic keratinocytes (OR, 0.13; 95% CI, 0.02–0.90) (Table 6).

It is of note that seven of the eight (87.5%) patients included in the anti-ARS category were anti-PL-7+ and eight of the nine (88.9%) patients in the anti-cytoplasmic category were anti-ARS+.

3.1.8 | Nuclear antibodies

We found a statistically significant ($p < 0.05$) association between nuclear antibodies and a higher degree of vacuolar degeneration (OR, 10.9; 95% CI, 1.26–94.2), marked thickened epidermal basement membrane (OR, 10.0; 95% CI, 1.56–65.0), and increased pigment incontinence (OR, 4.66; 95% CI, 1.20–18.1) (Table 6).

3.2 | Binary variables

3.2.1 | Anti-TIF1-γ

We found a nonstatistically significant ($p = 0.045$) trend of increased neutrophils and anti-TIF1-γ antibodies whose 95% CI included the null hypothesis (OR, 11.0; 95% CI, 0.76–648.0) (Table 7).

3.2.2 | Anti-synthetase antibodies

There was a nonstatistically significant ($p = 0.040$) trend of absent ectatic vessels in samples positive for anti-ARS serology, as the 95% CI included the null hypothesis (OR, 0.16; 95% CI, 0.02–1.20) (Table 8).

3.2.3 | Nuclear antibodies

There was a statistically significant ($p < 0.05$) association between the presence of epidermal atrophy (OR, 6.20; 95% CI, 1.05–68.5) and nuclear antibodies (Table 8).

4 | DISCUSSION

This pilot study examines correlations between serotypes of DM and histopathologic findings, revealing several statistically significant features. Most prominently in our data, a coherent picture of anti-PL7

TABLE 7 Association between binary features and individual serotypes as determined by Fisher's exact test.

	Anti-TIF1-γ		
	OR	95% CI	*p*-value
Neutrophils	11.0	(0.76, 648.0)	0.045*

Note: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
Abbreviations: CI, confidence interval; OR, odds ratio.

TABLE 8 Association between binary features and grouped serotypes as determined by Fisher's exact test.

	Classification System 1								Classification System 2							
	Anti-synthetase antibodies			Myositis-specific antibodies			Myositis-associated antibodies		Cytoplasmic antibodies			Nucleolar antibodies			Nuclear antibodies	
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Epidermal atrophy	0.18	(0.00, 1.68)	0.121	3.51	(0.78, 17.6)	0.094	0.71	(0.14, 3.20)	0.740	0.38	(0.03, 2.49)	0.437	0.00	(0.00, 1.64)	0.136	6.20	(1.05, 68.5)	0.038*
Ectatic vessels	0.16	(0.02, 1.20)	0.040*	6.30	(0.68, 315)	0.109	1.05	(0.17, 8.03)	1.00	0.20	(0.03, 1.47)	0.065	1.04	(0.08, 58.3)	1.00	3.91	(0.61, 30.8)	0.109

Note: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.
Abbreviations: CI, confidence interval; OR, odds ratio.

DM and anti-Ro/SSA DM emerges. Some features of anti-TIF1-γ DM and anti-Mi-2 DM are also found. Lastly, findings that may help distinguish between anti-cytoplasmic versus antinuclear antibodies are identified. Particularly because anti-PL7 is associated with severe, refractory ILD, anti-Mi-2 is associated with a positive prognosis, and anti-TIF1-γ is associated with a significantly elevated risk of malignancy, these results may be useful in diagnosis and prognostication.

Anti-PL-7 was significantly associated with minimal vacuolar degeneration, fewer necrotic keratinocytes, and minimally thickened epidermal basement membranes. A similar pattern was seen in the anti-ARS antibody group, in addition to a nonstatistically significant trend of absent ectatic vessels. The cytoplasmic serotype group also displayed a significant association with decreased vacuolar degeneration and fewer necrotic keratinocytes, but not with minimally thickened epidermal basement membrane. This could be either because the histologic features of anti-ARS or anti-cytoplasmic serologies are highly similar to those of anti-PL7, or because, in our cohort, most patients comprising the anti-ARS and cytoplasmic serotype groups were anti-PL-7+. While our pilot study was not designed to distinguish between these possibilities, future research is warranted to determine if histopathologic features of anti-PL7 DM are distinct from those of all anti-ARS and anti-cytoplasmic serologies.

Importantly, anti-PL-7 DM has been found to be associated with ILD, a disease with a 5-year survival worse than Stage III melanoma.[1] Though all the anti-ARS antibodies may present with ILD, previous literature suggests anti-PL-7 presents predominantly with ILD, in contrast to other anti-ARS antibodies with more varied phenotypes.[19] The minimal extent of histologic changes in the skin found in anti-PL-7+ patients in our study may reflect the fact that the primary disease process is targeted toward tissues of the lung. Moreover, our findings indicate that the subtlety of the histologic features in the skin may be misleading; they should not serve as indicators of mild skin disease, but rather as harbingers of serious pulmonary complications.

There is a sound biological basis for the histopathologic pattern identified in the anti-PL-7 cases. In general, when significant interface change occurs, keratinocytes become necrotic, and there can be injury to the basement membrane. The latter undergoes repair and reduplication, resulting in a thickened appearance. Therefore, it follows that the minimal vacuolar changes seen in anti-PL-7 patients would be accompanied by minimal necrotic keratinocytes and minimal (or absent) thickening of the basement membrane. Typical examples of DM with anti-PL7 antibodies are depicted in Figure 1.

Anti-TIF1-γ DM is frequently associated with visceral malignancy.[2] In our study, the histopathology of anti-TIF1-γ was characterized by frequent necrotic keratinocytes and pigment incontinence (Figure 2). These findings may be attributed to the severity of inflammation and interface change in these patients. An increased number of neutrophils was also noted, though not found to be statistically significant. Future studies could investigate whether the combination of frequent necrotic keratinocytes, pigment incontinence, and possibly increased neutrophils, irrespective of other findings, can be a clue to visceral malignancy in DM.

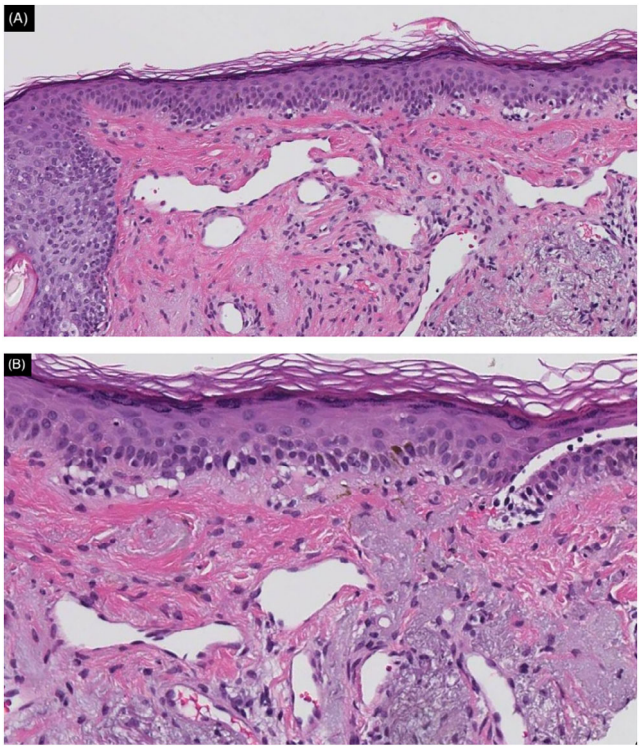

FIGURE 1 A typical anti-PL7 case of dermatomyositis (DM) (Patient 10). (A) There are prominent telangiectasias and abundant solar elastosis, whereas the degree of vacuolar interface change is fairly subtle on hematoxylin and eosin (H&E) stain at ×100 magnification. (B) Close scrutiny at high magnification reveals rare, focal remnants of necrotic keratinocytes, and essentially no thickening of the basement membrane on H&E stain at ×200 magnification. Subtle interface changes, rare necrotic keratinocytes, and minimal basement membrane thickening were typical of DM cases associated with anti-PL7 serology in our series.

Anti-Mi-2 serology was also associated with pigment incontinence. While pigment incontinence is reflective of inflammatory damage, to the best of our knowledge, it has not been pathologically linked to anti-Mi-2 antibodies before. Anti-Mi-2 and its correlated histology has been studied in myositis biopsies, and it is significantly associated with increased inflammation compared to other autoantibody groups.[20] It is possible that, similar to the process in muscle, anti-Mi-2 serotypes have a vigorous cutaneous inflammatory infiltrate, which eventuates in the pigment incontinence we observed. Notably, anti-Mi-2 serology is associated with a positive prognosis and has been shown to respond to rituximab.[20] The histological finding of pigment incontinence may point physicians toward this potential therapy, at least in select cases.

There was also a significant association between MSAs and pigment incontinence. Further research is warranted to assess if pigment incontinence is a sensitive marker for MSA DM or if there are individual serotypes where this pattern is most prominent.

We found that anti-Ro/SSA was characterized by a high degree of vacuolar degeneration (Figure 3). This is consistent with histopathologic findings in other cutaneous diseases with anti-Ro/SSA positivity, including neonatal cutaneous lupus erythematous, a disease mediated by transplacental transfer of maternal anti-Ro/SSA in which vacuolar alterations at the dermoepidermal interface is particularly prominent.[21,22] Anti-Ro/SSA has been found to be an independent risk factor for high relapse in patients with DM and polymyositis.[23] If the correlations found in the present study are further validated in future studies, a dermatopathologist may be able to suggest DM cases at risk for relapse by the degree of vacuolar degeneration change.

Nuclear antibodies were significantly associated with degree of vacuolar degeneration, thickened epidermal basement membrane, pigment incontinence, and epidermal atrophy in our study. Anti-Mi-2 is associated with a higher frequency of V-sign, Shawl sign, and increased muscle weakness, as well as lower rates of ILD and malignancy, good treatment response, and favorable prognosis.[24] Anti-SAE can present with severe cutaneous disease and commonly presents initially with skin manifestations alone.[25] As mentioned above, anti-TIF1-γ often presents with cancer-associated DM.[26] Despite these clinical differences, these serotypes share a common family of antibody targets. Our data reveals that they also share similar degrees of vacuolar change, basement membrane thickening, pigment incontinence, and epidermal atrophy. Future studies could investigate whether these similarities reflect a shared pathogenesis.

Notably, nearly one-third of the skin biopsies examined in this study revealed a small number of eosinophils. Previous research on the presence of eosinophils in DM has yielded mixed results. Some studies have reported essentially absent eosinophils in patients with DM, while others, including our own, have found that a substantial fraction have at least some eosinophils.[27,28] It may be worthwhile for future studies to revisit the question of whether a limited population of eosinophils can be used to exclude DM.

The strengths of this pilot study include the use of several dermatopathologists independently reviewing the skin biopsy samples. Our sample also contained patients with a range of myositis antibodies. However, given that DM is a rare disease, and this study was performed at a single institution, there were low patient numbers in the individual serology groups. This low cohort size limits the generalizability of our findings, as it is challenging to control for all potential confounding factors (e.g., age, sex, ethnicity, disease duration, previous treatments) that might influence the outcomes. Larger, more diverse studies—with patients from different geographic locations—are needed to validate these preliminary data. This validation is especially critical for a heterogeneous condition like DM, where symptoms and serological markers can vary widely.

Additionally, a limitation of the study is the lack of diversity of skin tones in the sample size as a result of the catchment area patient demographics. The average Fleiss kappa across all features assessed was below 0.4, indicating fair agreement.[29] Since this study shows significant findings despite some inter-rater variability, the results could be generalizable. In real world practice, a degree of variability will occur, and the results remained significant even when multiple dermatopathologists reviewed the cases. This pilot study points to the existence of associations between histologic features in the skin and serology in DM patients, and further research with greater sample

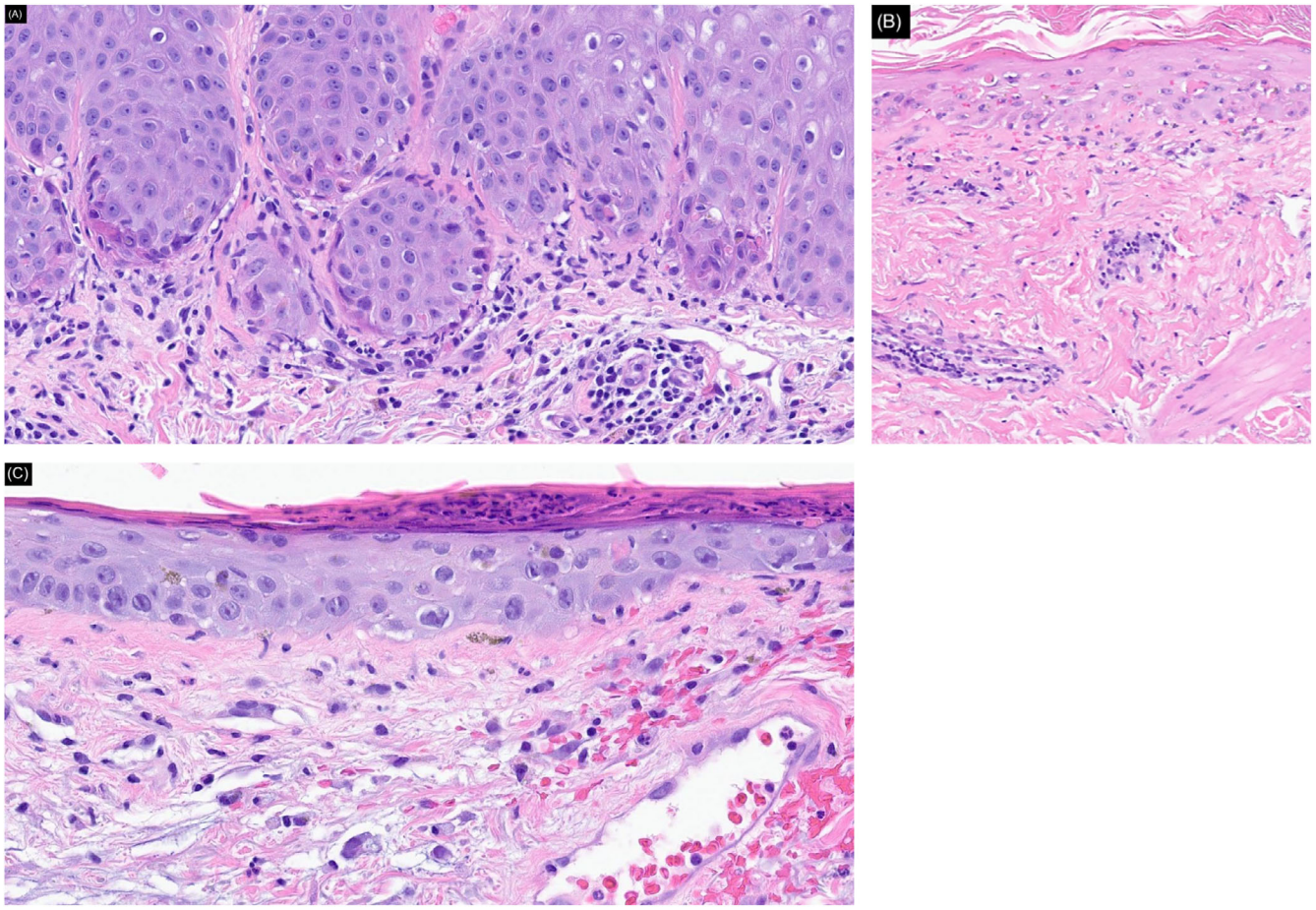

FIGURE 2 Cases of anti-TIF1-γ dermatomyositis (DM) (Patient 23 [A, B], Patient 29 [C]). (A) Necrotic keratinocytes, though not florid in absolute terms, are encountered more often than in other serotypes of DM, hematoxylin and eosin (H&E) stain, ×200. (B) Necrotic keratinocytes seen in another skin biopsy from the same patient, H&E stain, ×100. (C) Rare scattered neutrophils are also more commonly found than in other serotypes of DM, H&E stain, ×230.

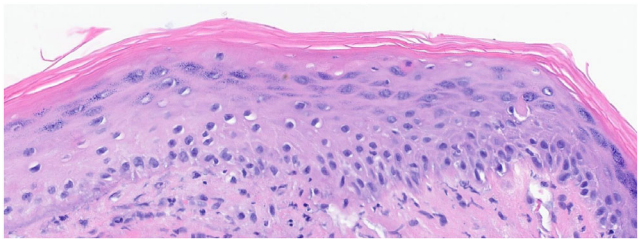

FIGURE 3 A case of anti-SSA dermatomyositis (Patient 19). Features typical of this serotype include a more pronounced degree of vacuolar interface change than that seen in other serotypes. Hematoxylin and eosin stain, ×200.

sizes is warranted to validate and expand upon the present findings. Furthermore, establishing a clinicopathologic correlation between serotype and skin biopsy findings can lead to an earlier diagnosis and enhanced patient care. For example, in cases of low myositis antibody titers, the myositis antibody-specific patterns observed on skin biopsy can support the existence of a specific DM phenotype. Perhaps most importantly, different serotypes may indicate different underlying biology, with specific histologic patterns; identifying those patterns may pave the way for more targeted therapies in the future.

5 | CONCLUSIONS

Our pilot study identifies several statistically significant associations between histopathologic features and DM serotypes. Anti-PL7 DM is characterized by minimal vacuolar change, few necrotic keratinocytes, and minimal or absent basement membrane thickening. Anti-Ro/SSA DM features indicate a high degree of vacuolar degeneration. Pigment incontinence and necrotic keratinocytes are positively associated with anti-TIF1-γ. Anti-Mi-2 is associated with pigment incontinence, as is MSA DM. Antinuclear DM is associated with prominent vacuolar change, thickened basement membranes, pigment incontinence, and atrophy. Further research may uncover additional clinicopathologic correlations that could improve the prognosis and therapeutic decision-making in the treatment of patients with DM.

AUTHOR CONTRIBUTIONS

Raven Bennett, Mirjana Stevanovic, Katherine Bradley, and Aravindhan Sriharan wrote and edited the manuscript. Alvaro J. Ramos-Rodriguez, Iman Salem, Katherine Bradley, and Emma L. Hodson edited the manuscript. Mirjana Stevanovic performed the statistical analysis. Jason R. McFadden organized the data. Raven Bennett, Jason R. McFadden, Katherine Bradley, and Advaita S. Chaudhari conducted the chart review. Aravindhan Sriharan, Shaofeng Yan, Shabnam Momtahen, Robert E. LeBlanc, Jeffrey M. Cloutier, and Alvaro J. Ramos-Rodriguez reviewed the skin biopsy slides for histopathologic features. Raven Bennett, Jason R. McFadden, Katherine Bradley, Advaita S. Chaudhari, David G. Grand, Emma L. Hodson, and Iman Salem provided technical support to the dermatopathologists who reviewed the skin biopsy slides.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

ETHICS STATEMENT

The study was IRB approved under the IRB number 00017556.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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